

Sabin IPV Process Development

Polio Immunization: Moving Forward
NIH, Bethesda, USA

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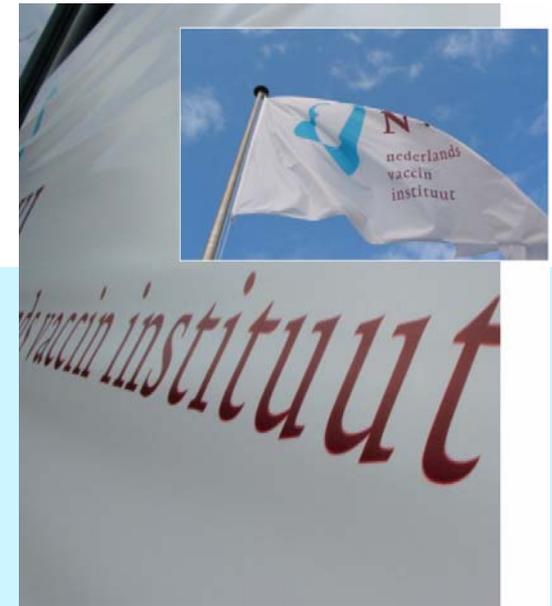
Introduction to NVI

Established in 2003 by a merger of:

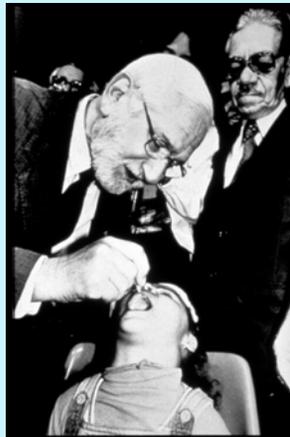
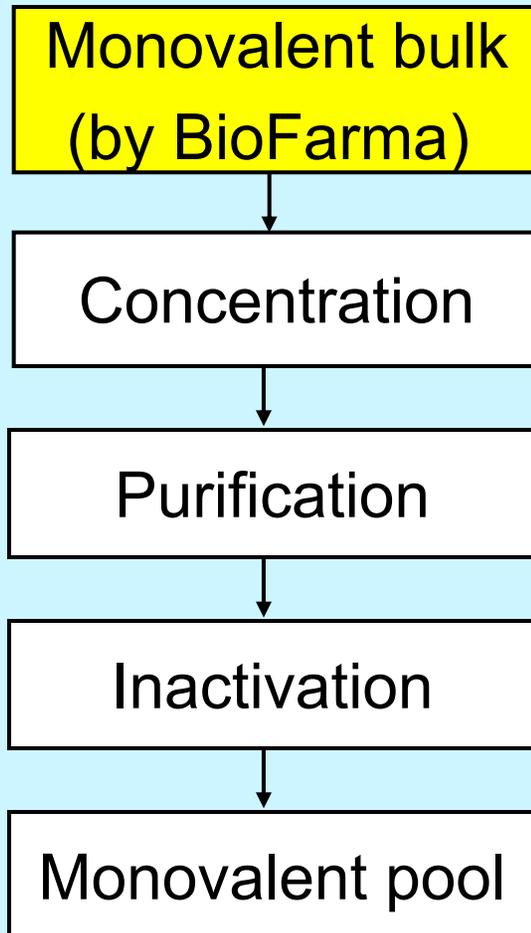
- RIVM Vaccine Division &
- SVM

NVI is a government-based vaccine institute
Independent from commercial vaccine suppliers

Over 100 years of vaccine experience in the public domain



Lab-Scale Sabin-IPV Purification Scheme



Collaboration initiated by WHO:

BioFarma: Sabin OPV bulks

JPRI: Supply of Master Seeds

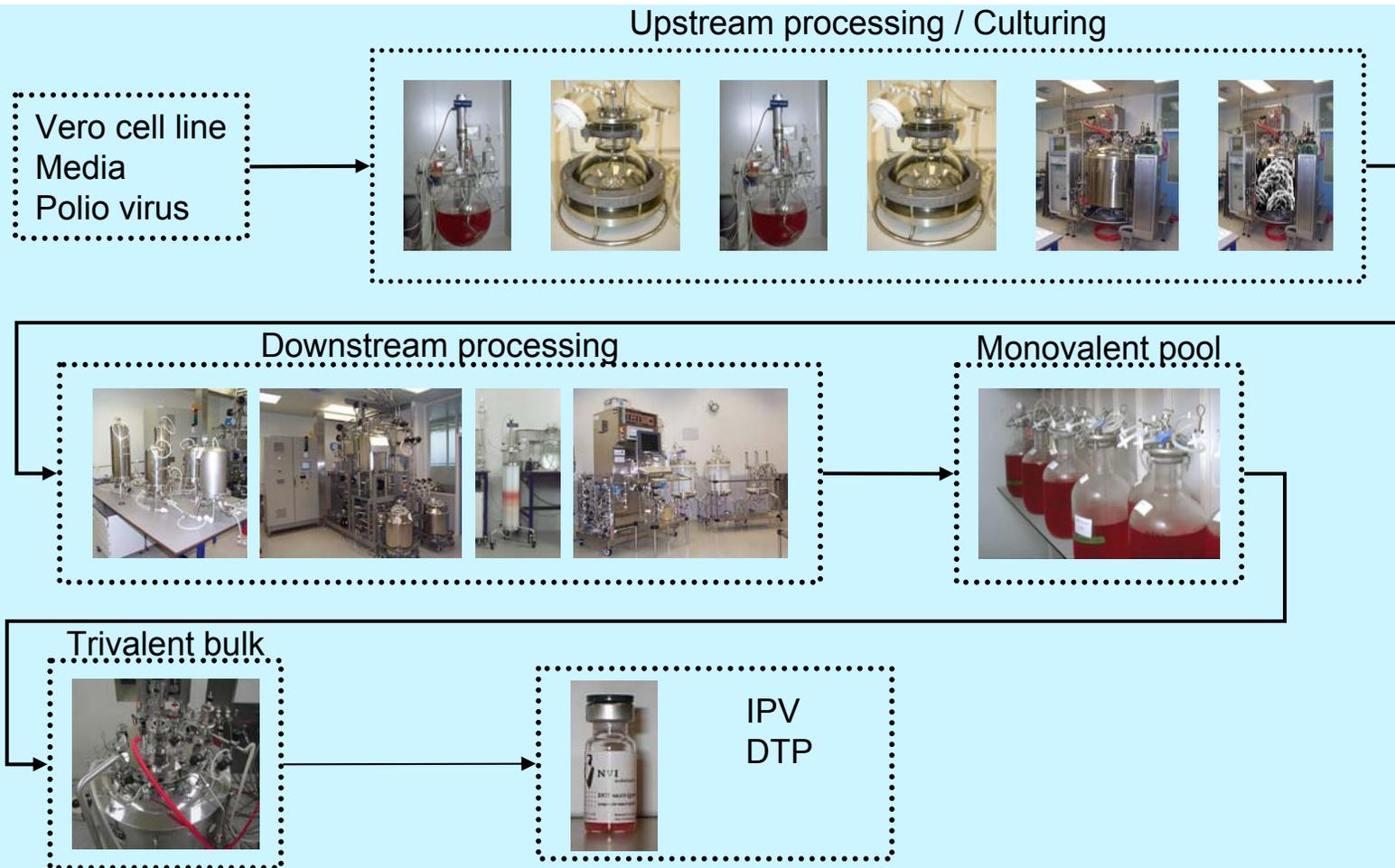
NVI: Pharmaceutical Development

NIBSC: Norms & standards

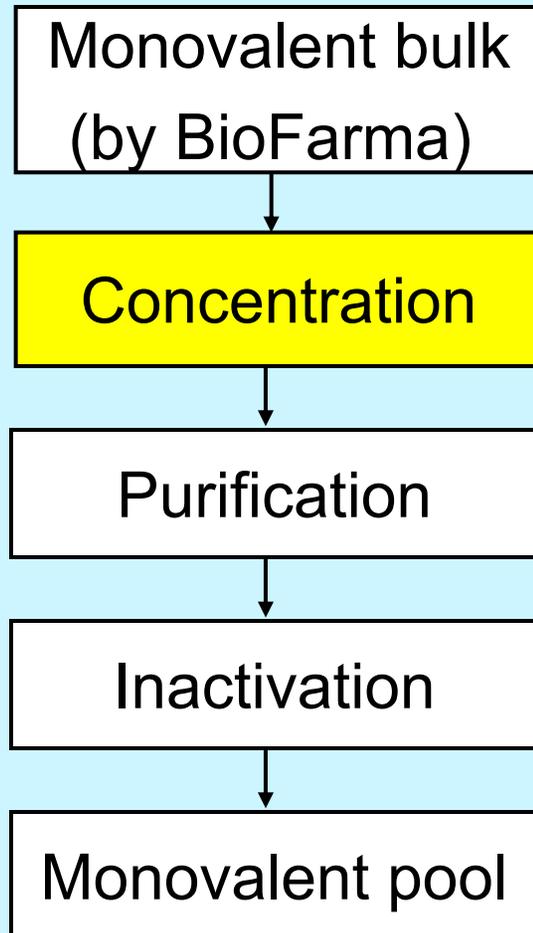
Proof-of-principle project:

Preparation of purified trivalent inactivated Sabin-IPV based on the current NVI IPV-process

Current wt-Polio (IPV) production scheme



Lab-Scale Sabin-IPV DSP: Concentration



- 3 x Monovalent Sabin OPV (3 sub-types)
- 2-step Concentration process
- Performed by Ultra-Filtration
- Concentration factor is approx. 500 times

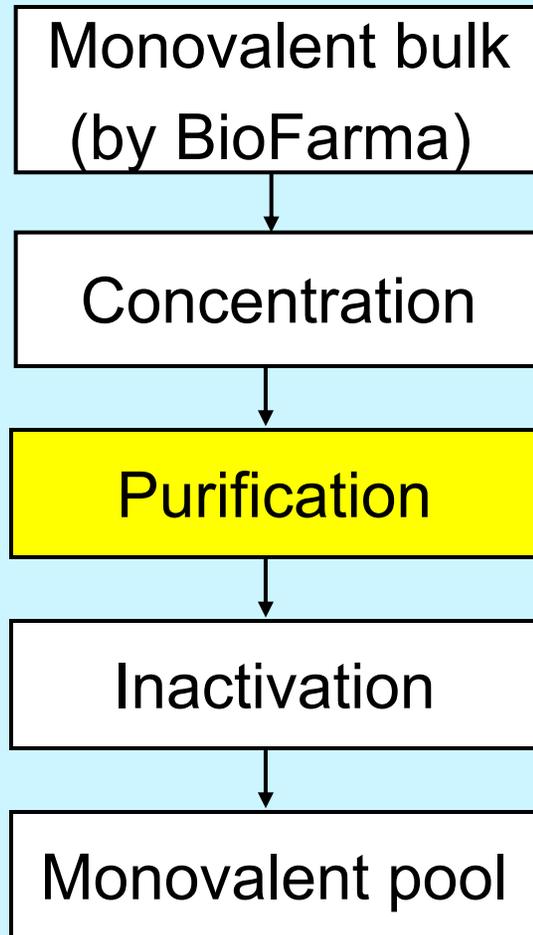


Overview concentration

Sabin Type	Estimated* D-ag at start [e-DU/ml]	Start Volume [ml]	End Volume [ml]	Concentration factor [-]	Estimated* D-ag at end [e-DU/ml]	D-ag* Recovery [%]
1	51	10438	22	485	23782	97
2	7	19800	35	566	3705	100
3	27	10275	24	437	n.a.	n.a.

*) Based on an estimation by a fast D-antigen ELISA test used for Process Dev. only

Lab-Scale Sabin-IPV DSP: Purification

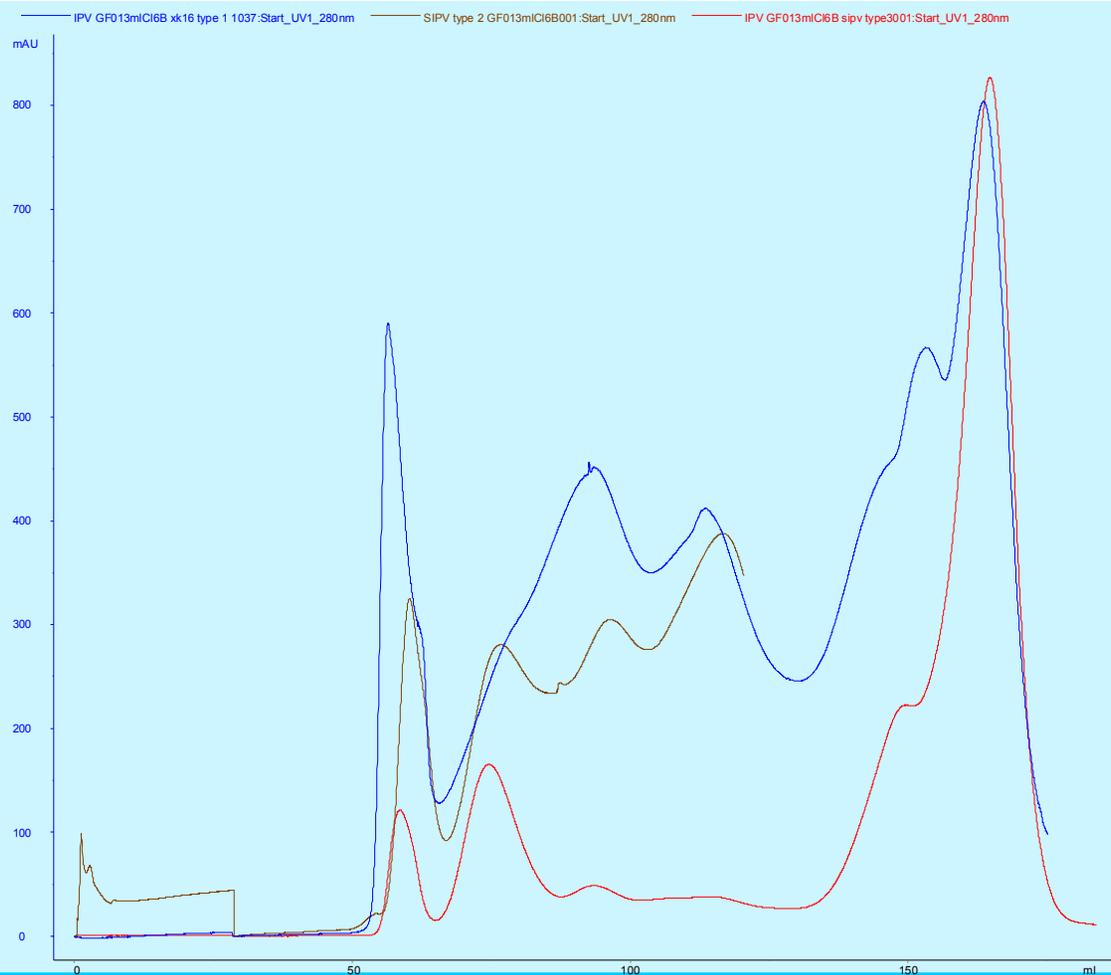


2-step Column Chromatography:

- Size Exclusion Chromatography (SEC)
 - **Remove aggregates & small-size molecules**
- Ion Exchange Chromatography (IEX)
 - **Remove host cell proteins**



Purification step 1: Size Exclusion Chromatography (Column matrix: Sepharose CL-6B)



- Blue : Sabin Type 1
- Brown : Sabin Type 2
- Red : Sabin Type 3

Sabin	A260/ A280	Recovery [%]
T1	0.71	n.a.
T2	1.18	54
T3	1.70	70

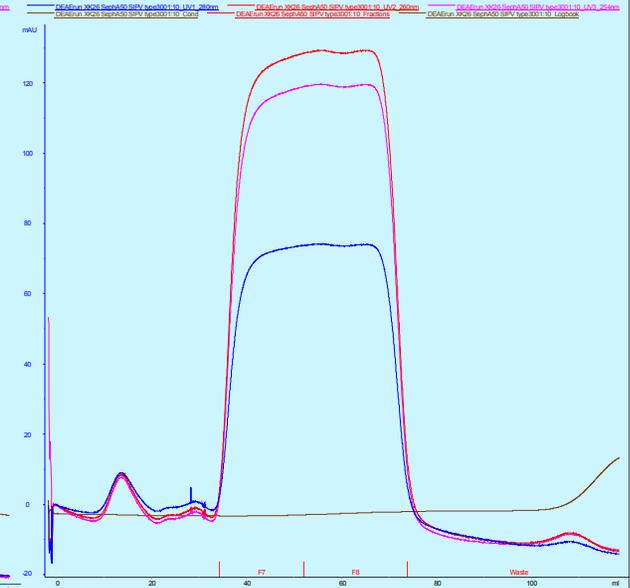
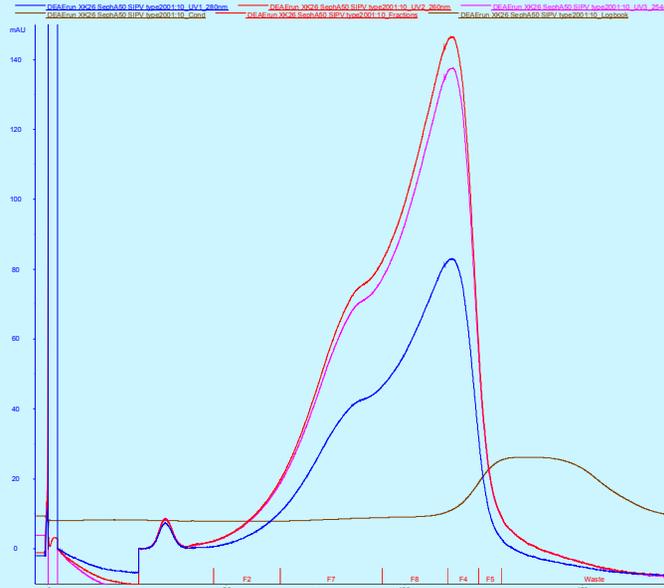
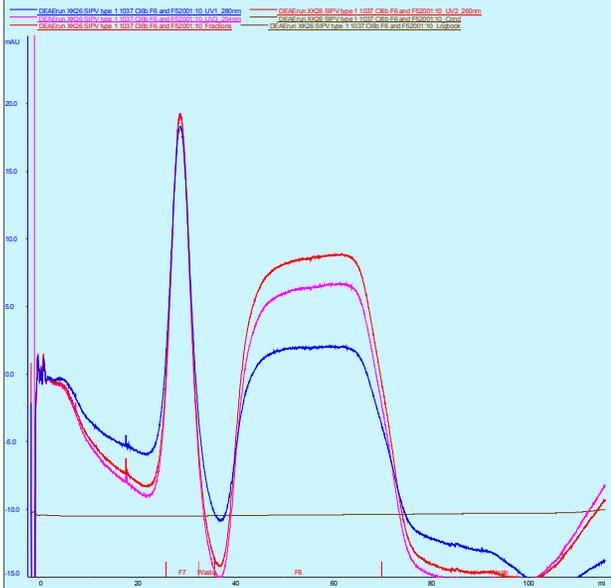
wt-IPV A260/A280 ratio = 1.21 ± 0.22

Purification step 2: Ion Exchange Chromatography (Column matrix: DEAE Sephadex A50)

Type 1

Type 2

Type 3



Ratio A260/280 = 1.16

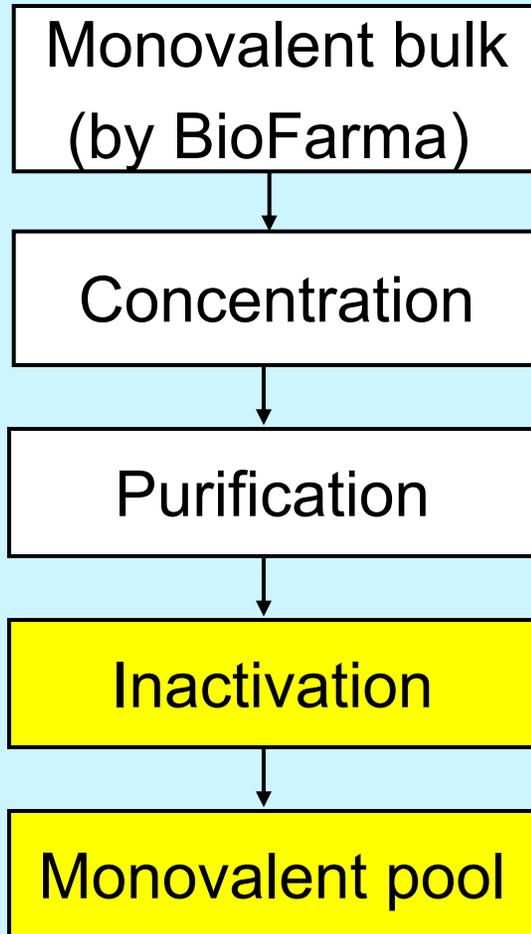
Ratio A260/280 = 1.75

Ratio A260/280 = 1.60

wtIPV 260/280 ratio = 1.74 ± 0.14

Recovery $\approx 60 - 70\%$

Lab-Scale Sabin-IPV DSP: Inactivation



Inactivation by using formaldehyde (3 mM):

- Incubate 6 – 8 days at 37°C
- Sterile filtration (0.22 μm) to remove aggregates
- Incubate 6 days at 37°C



Storage:
max. 3 years at 2 – 8 °C

Process recovery for inactivated bulkproduct

Sabin Type	Volume [ml]	Estimated* D-ag conc. [e-DU/ml]	Process recovery [%]
1	50	201	39
2	52	217	31
3	61	989	38

*) Based on an estimation by a fast D-antigen ELISA test used for Process Dev. only

Average process recovery for wt-IPV: 45%

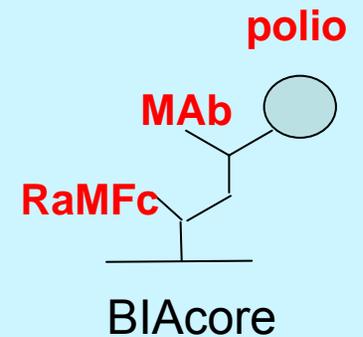
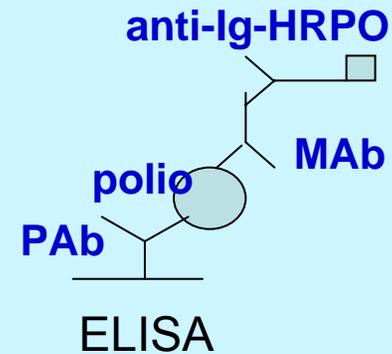
Characterization & Future plans

- Virus titration
- D-ag content (ELISA & Biacore)
- Particle size distribution
- Purity (SDS-PAGE)
- Adsorption to glass
- Thermostability (& C-ag content)
- Formulation
- Dose finding (Rat potency test)
- Potency testing (in Rats)

D-ag content (ELISA & BIAcore)

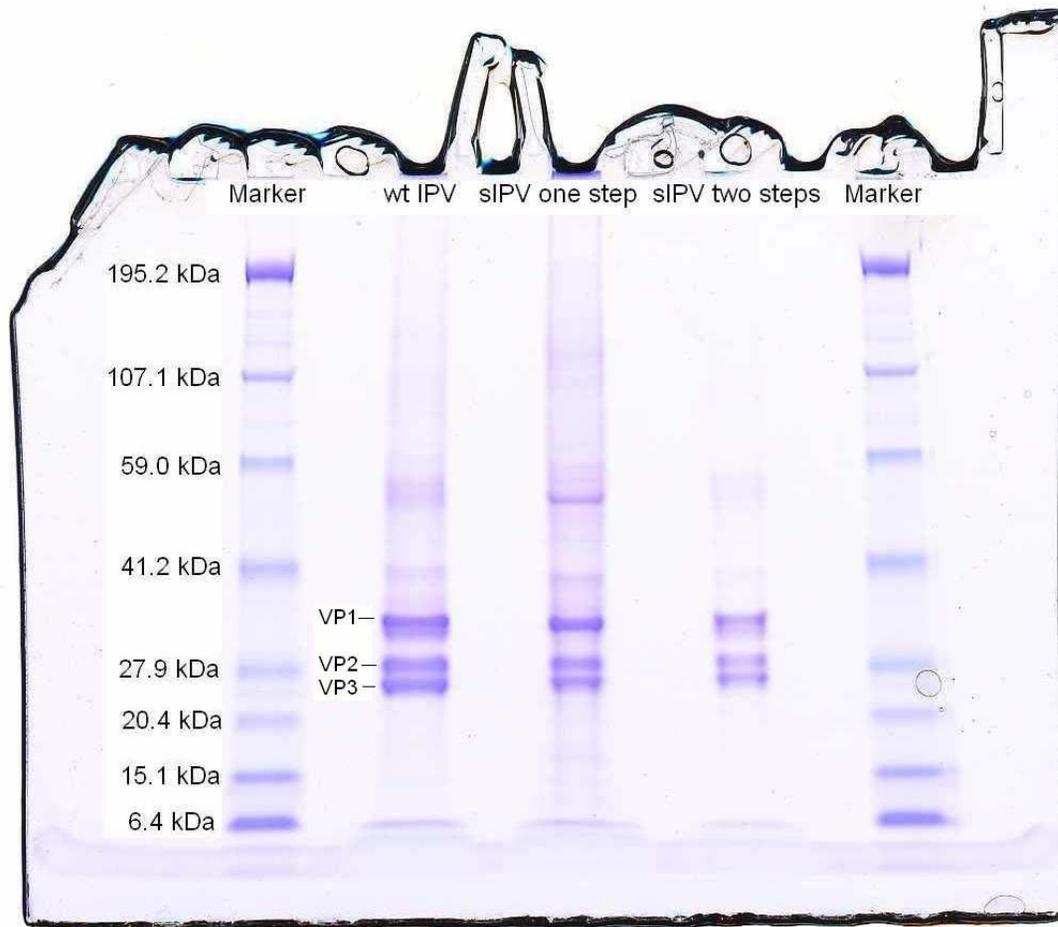
Type 1	BIAcore Site 2,3 [DU/ml]	ELISA Site 2,3 [DU/ml]	Ratio BIAcore/ELISA	Virus conc. [µg/ml]	Spec. Antigenicity [DU/µg virus]
s-IPV purified	1404	153	9.2	n.a	n.a.
s-IPV inactivated	81	51	1.6	6	9
wt-IPV inactivated	1655	1994	0.8	87	23

Type 2	BIAcore Site 2,3 [DU/ml]	ELISA Site 2,3 [DU/ml]	Ratio BIAcore/ELISA	Virus conc. [µg/ml]	Spec. Antigenicity [DU/µg virus]
s-IPV purified	246	187	1.3	n.a.	n.a.
s-IPV inactivated	192	200	1.0	10	21
wt-IPV inactivated	1034	1074	1.0	67	16



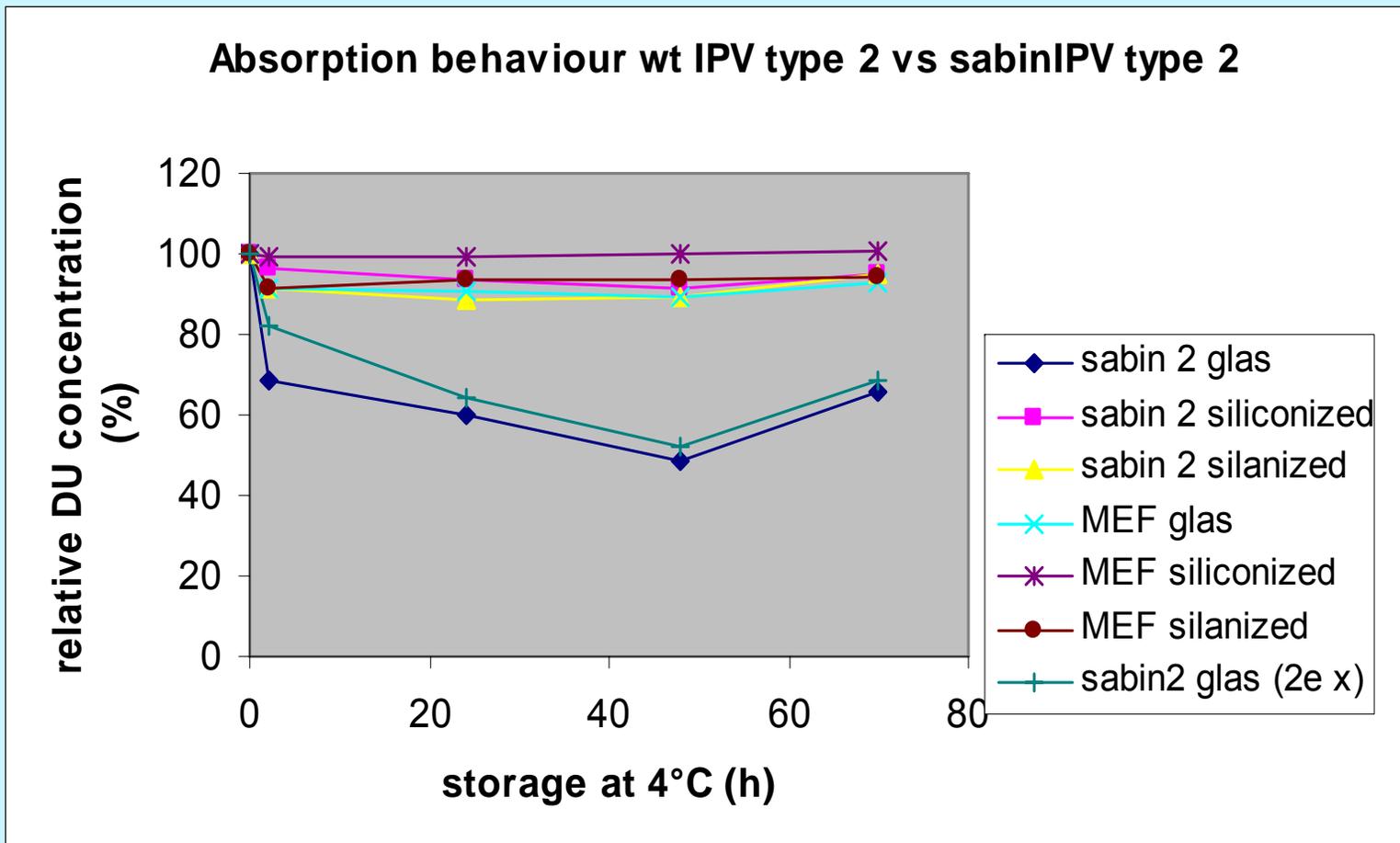
Type 3	BIAcore Site 2,3 [DU/ml]	ELISA Site 2,3 [DU/ml]	Ratio BIAcore/ELISA	Virus conc. [µg/ml]	Spec. Antigenicity [DU/µg virus]
s-IPV purified	715	690	1.0	n.a.	n.a.
s-IPV inactivated	526	544	1.0	17	31
wt-IPV inactivated	994	1121	1.0	81	14

Purity



SDS-PAGE type 2
(1st trial on lab-scale)

Adsorption to glass



Acknowledgements / Questions?

- Aart van 't Oever
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